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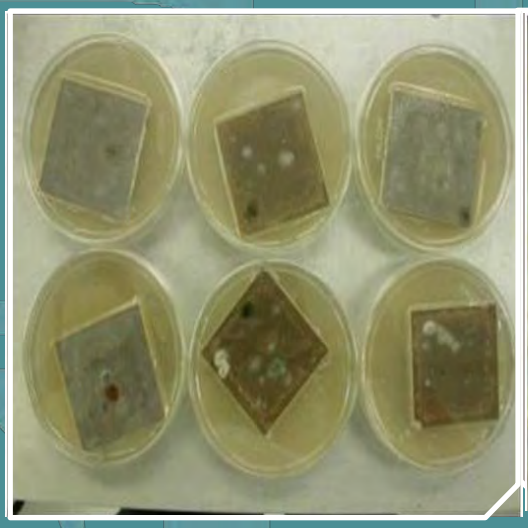
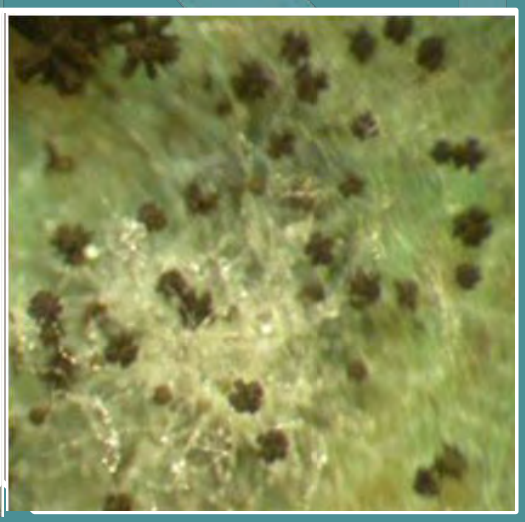
United States
Environmental Protection
Agency

Microbial Resistant Test Method Development





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Microbial Resistant Test Method Development

By

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Abstract

Humans spend most of their time in the indoor environment. Environmental analysis of the quality of indoor air has and continues to be an important research topic. A major component of the aerosol in the indoor environment consists of biological particles, called bioaerosols. A major fraction of these bioaerosols are fungal in origin. These fungal organisms have been shown to cause adverse health effects ranging from mild headaches to cases of idiopathic pulmonary hemosiderosis in infant children. To prevent fungal organisms from growing in the built environment many companies have developed and marketed microbial resistant building products. These companies have taken different strategies to produce microbial resistant products, including removing fungal growth substrates to adding antimicrobial chemicals into the final product. The aim of this study was to develop a quantitative antimicrobial testing method coupled to product volatile organic compound (VOC) offgassing analysis. This coupled microbial/chemical analysis is holistic and produces a true measure of the effectiveness of the product as well as information on VOC production. The developed test method was used to test three different classes of building materials for both microbial resistance and VOC offgassing. Important findings included extending the testing from 6 weeks to 12 weeks which allowed ubiquitous microbes to grow indicating the importance of the more comprehensive testing duration. Additionally, quantitative analysis removed all uncertainty in determining the microbial resistance of a specific product.

Once the test method was completed, this project expanded to evaluate currently utilized microbial (fungal) resistant testing methodologies as they are applied to gypsum products. Currently there are numerous methods that allow manufacturers to test for microbial resistance. Each of these methods is qualitative in nature allowing for results to be interpreted differently by various laboratories. Five testing methods were identified and chosen to compare following a literature search. We obtained detailed documents explaining the specific steps for completing the testing methods as they are meant to be utilized, and how the results are to be interpreted. Following our completion of these tests, the results show that the more stringent quantitative method removed all ambiguity from the analysis, as well as allowing for a more complete duration of testing lasting 12 weeks. While all of the tests are appropriate for their individual purposes, the quantitative test method developed and described herein works for a multitude of different microbial resistant products and product classes.

This report covers a period from September, 2011 through March, 2015.

Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the Nation's land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, EPA's research program is providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

The National Risk Management Research Laboratory (NRMRL) within the Office of Research and Development (ORD) is the Agency's center for investigation of technological and management approaches for preventing and reducing risks from pollution that threaten human health and the environment. The focus of the Laboratory's research program is on methods and their cost-effectiveness for prevention and control of pollution to air, land, water, and subsurface resources; protection of water quality in public water systems; remediation of contaminated sites, sediments and ground water; prevention and control of indoor air pollution; and restoration of ecosystems. NRMRL collaborates with both public and private sector partners to foster technologies that reduce the cost of compliance and to anticipate emerging problems. NRMRL's research provides solutions to environmental problems by: developing and promoting technologies that protect and improve the environment; advancing scientific and engineering information to support regulatory and policy decisions; and providing the technical support and information transfer to ensure implementation of environmental regulations and strategies at the national, state, and community levels.

The Indoor Environment Management Branch (IEMB), a research unit belonging to the Air Pollution Prevention Control Division (APPCD) within NRMRL is tasked with developing a better understanding of the quality of indoor air and its relationship to different emissions sources including home furnishings, building products, building mechanical systems, and building design. These research findings are communicated to EPA regions, the Office of Air and Radiation, architects, building managers, contractors, and the general public so that they can make educated decisions on the materials and building systems which improve indoor air quality. This project directly addresses building material selection and usage as it relates to biological contamination and its adverse effects on indoor air quality.

Cynthia Sonich-Mullin, Director
National Risk Management Research Laboratory

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Acronyms/Abbreviations/Definitions

AATCC	American Association of Textile Chemists and Colorists
ACH	air changes per hour
ADQ	audit of data quality
AIHA	American Industrial Hygiene Association
ASTM	American Society for Testing and Materials
a_w	water activity
CC	Culture Collection
CFU	colony forming unit
DNPH	2,4-dinitrophenylhydrazine
DQO	data quality objective
EPA	U.S. Environmental Protection Agency
ERH	equilibrium relative humidity
ESTE	Environmental and Sustainable Technology Evaluation
ETV	Environmental Technology Verification
g	gram(s)
GC/MS	gas chromatography/mass spectrometry
HVAC	Heating Ventilation Air Conditioning
ISO	International Organization for Standardization
MC	moisture content
ML	microbiology laboratories
mL	Milliliter
ML SOP	microbiology laboratory standard operating procedure
NERL	National Exposure Research Laboratory
OSWER	Office of Solid Waste and Emergency Response
QA	quality assurance
QAM	quality assurance manager
OAR	Office of Air and Radiation
ORD	Office of Research and Development
QAPP	quality assurance project plan
QC	quality control
QMP	quality management plan
RH	relative humidity
RTI	Research Triangle Institute (RTI International)
SDA	Sabouraud dextrose agar
sec	second(s)
SOP	standard operating procedure
SPE	solid phase extraction
spp	species
t	temperature
TLV	threshold limit value
TOP	technical operating procedure
T/QAP	test/quality assurance plan
TSA	technical system audit
TVOC	total volatile organic compounds
VOC	volatile organic compounds
µg	microgram(s)
UL	Underwriters Laboratories
µm	micrometer(s)

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1.0 Problem Definition/Background Information

Fungal growth and the resulting contamination of building materials is a well-documented problem, especially after the reports from New Orleans and the US Gulf Coast post Hurricane Katrina. However, contaminated materials have been recognized as important indoor fungal reservoirs for years. For example, contamination with fungi has been associated with a variety of materials including carpet, ceiling tile, gypsum board, wallpaper, flooring, insulation, and heating, ventilation and air conditioning components[1,2,3,4].

Exposure to fungi may result in respiratory symptoms of both the upper and lower respiratory tract such as allergy and asthma[5]. Everyone is potentially susceptible. However, of particular concern are children with their immature immune systems and individuals of all ages that are immunocompromised[6,7].

One approach to limiting exposure is to reduce the levels of fungi in the indoor space. For some sensitive individuals, limiting exposure through avoidance is an effective control method; however, avoidance is not always possible or practical. The investigation, development, and application of effective source controls and strategies are essential to prevent fungal growth in the indoor environment. Mold resistant building material is a potentially effective method of source control.

A building is not a sterile environment, nor should it be. However, a building may serve as a reservoir for microorganisms. While many different types of microorganisms occupy indoor spaces, it is well-recognized that fungi can colonize and amplify on a variety of building materials if sufficient nutrients and moisture are present. These contaminated materials are known to be important indoor reservoirs. Fungal growth on natural and fabricated building materials can be a major source of respiratory disease in humans. Some common environmental fungi that have been isolated from contaminated materials include *Acremonium spp., Alternaria spp., Aspergillus spp., Chaetomium spp., Cladosporium spp., Epicoccum spp., Fusarium spp., Penicillium spp., Stachybotrys spp.,* and *Trichoderma spp.*

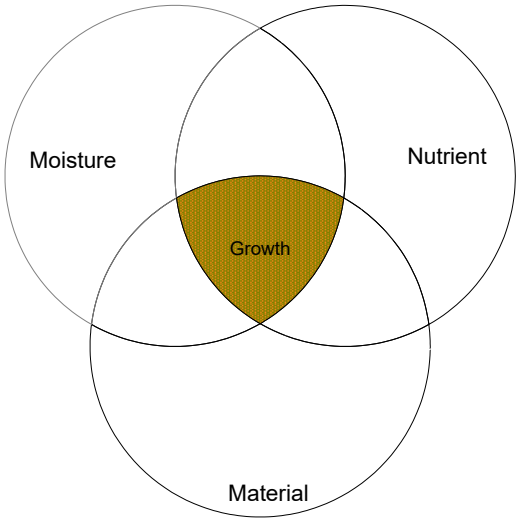

Figure 1. Diagram illustrating the conditions required for fungal growth on a material.

Figure 1 illustrates the combination of moisture and nutrients required for microbial growth on a material. Sufficient nutrients for growth may be provided by the material itself or through the accumulation of dust on or in the material. When sufficient nutrients are available, the ultimate determinate for microbial growth is availability of water. The more hygroscopic a material is, the more impact on the overall hygroscopicity the surface treatments may have.

According to the Lawrence Berkeley National Laboratories, improving buildings and indoor environments could reduce healthcare costs and sick leave and improve worker performance, resulting in an estimated productivity gain of $30 billion to $150 billion annually[8]. The Department of Energy further estimated the potential decrease in adverse health effects from improvements in indoor environments to be 10 percent to 30 percent for infectious lung disease, allergies and asthma; and 20 percent to 50 percent for Sick Building Syndrome symptoms[1]. For the United States, the corresponding annual healthcare savings plus productivity gains could be:

- $6 billion to $19 billion from reduced lung disease,
- $1 billion to $4 billion from reduced allergies and asthma,
- $10 billion to $20 billion from reduced Sick Building Syndrome symptoms,
- $12 billion to $125 billion from direct improvements in worker performance unrelated to health[8].

The indoor environment is an important area of research. The past twenty years have led to the recognition that building dampness is an important factor in the health of people that live and work in an indoor environment [2-4]. Furthermore, it is now recognized that the principal biological organisms responsible for the health problems in these environments are the fungi rather than bacteria and viruses [5]. Fungi (mold) may play an important part in the symptoms associated with sick building syndrome [6].

The research that follows addresses two specific characteristics of mold resistant building material: 1) mold resistance, and 2) emissions of VOCs (Volatile Organic Compounds) and aldehydes. Due to the multiple different testing methods publicly available, mold resistance is the critical measurement followed by product VOC offgassing. Therefore the emphasis of this research was on mold resistance. Emissions of VOCs and aldehydes are ancillary tests and may or may not be performed depending upon the relevance to the test material. Other characteristics, such as fire resistance, are important and should be considered by users of the products, but are beyond the scope of this research.

Mold resistance testing was performed following the guidelines outlined in ASTM (American Society for Testing and Materials) D6329-98 (2008)[9]. D6329 is a standard guide for developing methodology to evaluate the ability of indoor materials to support microbial growth using static environmental chambers. ASTM D6329 was developed as part of a more comprehensive project to apply indoor air quality engineering to biocontamination in buildings. One of the primary goals was to provide a scientific basis for studying indoor air biocontaminants. Available methods, including those from ASTM, AATCC (American Association of Textile Chemists and Colorists), and UL (Underwriters Laboratories), to evaluate the resistance of a variety of materials to fungal growth were surveyed at the initial stages of that project. Although the basic principals were similar, a major concern was the way growth on the different materials was evaluated. Although quantitative methods for inoculation were employed, none assessed growth as the endpoint quantitatively. The strategy was to improve upon D6329 by developing a new method that would provide a more quantitative endpoint for growth in a well-controlled environment and to improve repeatability and comparability. Additionally, it is well known that fungal organisms can be very slow growing. Therefore, extending the testing to 12 weeks would allow for any viable organisms to grow. The method has been successfully used to evaluate fungal resistance on a variety of materials including ceiling tiles, flooring, gypsum products, and HVAC (Heating Ventilation and Air Conditioning) duct materials [10,11,12,13].

A number of strategies have been employed to reduce the susceptibility of building materials to fungal growth and the subsequent spread of biological contaminants. Removal of growth substrates from building materials, or the incorporation of antimicrobial agents in the manufacturing of building products are two of those strategies. For example, there are several green building products readily available that have the potential to limit mold growth in the indoor environment. However, there is no quantitative testing method that generates results to guide consumers and building professionals on how to select or specify the best building products for their needs. The available test methods all rely on qualitative analysis of fungal growth which can lead to different microbial resistance ratings. However, the tests are too short to accurately determine fungal resistance, and they are also for specific product areas and not applicable to a broad range of building materials. This research was designed to fill this gap by developing a microbial resistant testing method suitable for multiple building materials, and testing the method against commonly utilized testing regimes. Multiple EPA offices including OAR (Office of Air and Radiation), ORD (Office of Research and Development), and OSWER (Office of Solid Waste and Emergency Response) and private sector organizations (e.g., the U.S. Green Building Council, and the Gypsum Association) have shown interest in standardizing the testing of their products. The resultant testing data will allow these organizations to assess the ability of these "green" products not only to improve the living conditions in the built environment, but to gauge if their increased use in construction will have positive impacts on the building material waste stream. The testing method includes the following: (1) mold growth, and (2) volatile organic compound (VOC) emissions. Established methods were used to form the basis of each of the developed test methods.

2.0 TEST METHODS AND PROCEDURES

Mold resistance testing was performed following the guidelines outlined in ASTM 6329[9]. The focus of method development was a quantitative end point removing ambiguity in interpreting fungal contamination levels, a 12 week total test duration allowing the slow growing fungal organisms the chance to grow, and the flexibility of the test method to be used on a multitude of different building product classes.

2.1 Test Organisms

Selecting the "correct" test organism is critical to any test, therefore selection criteria were developed. The selection criteria used to choose the appropriate test organisms for this study were:

(1) Reasonableness or likelihood of the test material being challenged by that particular organism when in actual use, and

(2) Extent to which the test material covered the range of ERHs (equilibrium relative humidities) needed and support the ERHs where fungal growth can occur.

Two fungi were used as test organisms, *Aspergillus versicolor* and *Stachybotrys chartarum*. Each of them met the criteria. *S. chartarum* requires high levels of available water to grow and has been associated with a number of toxigenic symptoms. *A. versicolor* is a xerophilic fungus and capable of growing at lower relative humidities. Both are from the RTI (Research Triangle Institute) culture collection (CC). *Stachybotrys chartarum* is CC #3075 and received from EPA NERL (National Exposure Research Laboratory). *A. versicolor* is CC #3348, and it is a field isolate. Prior to initiation of the testing, their identification was confirmed by standard techniques.

2.2 Static Chambers

Clear plastic desiccators served as the static environmental chambers. These chambers were purchased from Fisher Scientific, Pittsburgh, PA (product #08-647-47) and are readily available. The desiccators are sealed so there is no air exchange and the desiccators serve as good static chambers. A saturated-salt solution of potassium chloride was used to maintain the humidity of the 85% ERH chamber. Sterile water was used for the 100% ERH chamber. Temperature was externally controlled and maintained at room temperature (72º F =/- 2º F). Prior to use, the chambers were decontaminated and characterized making sure there were no cracks in the plastic, the feet were level, and the structural integrity of the seal was adequate. The ERH in each chamber was monitored with a hygrometer, Taylor model number 5565, (Taylor Precision Products, Las Cruces, NM) that was placed inside the chamber.

2.3 Sample Preparation and Inoculation

Small (at least 4 cm x 4 cm) replicate pieces of test mold resistant building products were prepared and inoculated. To minimize error and demonstrate reproducibility, five pieces of each sample type were processed on each sampling date. Because there were four test dates, a minimum of 20 pieces were prepared simultaneously. Each piece was placed on a separate labeled sterile Petri dish.

The fungi challenge suspensions were prepared by inoculating the test organism onto solid agar media, incubating the culture at room temperature until mature, wiping organisms from the surface of the pure culture, and suspending them in sterile 18-Mohm distilled water. The organism preparation was viewed microscopically to verify purity of spores (absence of hyphae). The test pieces were inoculated (usually with five 10 µL spots in an X configuration) by pipet onto the surface of the test piece and allowed to dry in the biosafety cabinet, an enclosed, ventilated laboratory workspace for safely working with materials contaminated with (or potentially contaminated with) biological organisms.

On each test date (including Day 0), the appropriate number of test pieces (5) were removed from the static chamber, each placed in approximately 30 mL sterile buffer (1X PBS), and extracted by shaking using a vortex or wrist action shaker. The extract was diluted if needed and plated on agar media to determine the numbers of CFU.

2.4 Test Design

The sample (small piece of the building material being tested) was cut aseptically with a razor blade into small pieces (at least 4 cm x 4 cm). The material was not autoclaved or sterilized in any way prior to inoculation. Therefore, in addition to the test organism inocula, any organisms naturally on both the top and bottom surfaces of the material had the opportunity to grow if conditions were favorable for growth.

The test organisms are inoculated by pipette directly onto the surface of each test piece in sufficiently high numbers (10^6 CFU) to provide an adequate challenge, but at a level that is realistic to quantify. The tests ran for 12 weeks. During the 12 week test period, data from four test dates, labeled Day 0, Week 1, Week 6, and Week 12 were evaluated. Day 0 samples provided the baseline inoculum level. A sufficient number of test pieces were inoculated simultaneously for all four test dates. All pieces for one material and one test organism were put in the same static chamber. The chambers were set to 100% equilibrium relative humidity (ERH) for the tests with *S. chartarum* and at 85% for *A. versicolor.* On each test date (including Day 0), five replicates of the test material pieces were removed from the chamber, each was placed separately in a container with sterile buffer (1X Phosphate Buffered Saline: 10 mM PO_4^{3-}, 137 mM NaCl, and 2.7 mM KCl), and extracted by shaking using a bench top vortex. The resulting suspension of eluted organisms was plated and microbial growth on materials was quantified by manually enumerating colony-forming units (CFUs), counting fungal colonies on the media plate.

The numbers of CFUs eluted on week 1, 6, and 12 were compared to the baseline at Day 0. The numbers of CFUs on each date are expressed as $\log_{10}$. The results are reported as the log change in CFUs between Day 0 and Week 1, Day 0 and Week 6, and Day 0 and Week 12.

2.5 Calculation of Mold Resistance

Changes in the numbers of CFU over time were quantified. The $\log_{10}$ number of CFUs from test date x were compared to the $\log_{10}$ number of CFU from Day 0 as follows:

$$\Delta\ log_{10}\ CFU = log_{10}\ CFU_{date\ x} - log_{10}\ CFU_{Day\ 0}$$

where:

Δ CFU = the change in log_{10} CFU between a test date (x) and Day 0
$log_{10}\ CFU_{date\ x}$ = number of CFU log_{10} on test date x
$log_{10}\ CFU_{Day\ 0}$ = number of CFU log_{10} on Day 0

The standard error of the means between the start date and the test date gives the statistical significance of the differences.

2.6 Formaldehyde and VOC Testing:

The main test for green building products is antimicrobial efficacy of the products. However, to make a more holistic test, analysis for product offgassing of formaldehyde and VOCs was included. Standard methods of VOC testing were utilized in the development of this test method. Briefly, two pieces of the sample material, contained in a 7"x7"x2" cradle of aluminum foil, were tested in a small (52.7 L capacity) emissions chamber maintained at 25 ºC and 50% relative humidity and subjected to an air exchange rate of 1 hr^{-1}. After equilibration of each sample for 6 hr^{14}, sequential samples for VOCs and carbonyls were collected from the chamber effluent for 20 and 120 minutes, yielding collection volumes of approximately 1.5 and 10 L for VOCs and 10 and 60 L for carbonyls[15]. In addition to the test material, replicate chamber blanks and the emission profile of a positive control material were collected.

All sample collections and analyses were conducted in accordance with RTI's AIHA quality manual guidelines[16]. VOC samples were collected on Carbopack B cartridges. A total of 100 ng of the internal standard, d8- toluene, was subsequently added to each cartridge by flash loading[17] prior to analysis by thermal desorption-GC/MS on a DB-5 column programmed from 40ºC - 225ºC at 5 ºC/min[18]. Calibration standards were prepared at two levels (3.5 µg; 6.9 ng) by flash loading of a 26-component VOC mixture (ethanol; isopropanol; acetone; dichloromethane; carbon disulfide; methyl –t –butyl ether; 2-butanone; 1,1,1-trichloroethane; 1-butanol; trichloroethene, 4-methyl-2-pentanone; toluene; hexanal; tetrachloroethene; m-xylene; n-nonane; 2-butoxyethanol; phenol; 1,2,4-trimethylbenzene; n-decane; 2-ethyl-1-hexanol; d-limonene; 1,2-dichlorobenzene; n-undecane; decamethylcyclosiloxane; n-dodecane) plus d9-toluene internal standard in methylene chloride onto Carbopack B. In addition to quantitation of the individual analytes, total VOCs (TVOC) were determined by summing the integrated peak areas in the samples and blanks between the retention times of hexane and hexadecane. Two specific analytes, 4-phenylcyclohexene and styrene, were sought in each sample. Neither compound was detected in the samples or blanks. All detected analytes were quantitated against the toluene peak in the standards. No mathematical correction for the blanks was performed. Carbonyl samples were collected on DNPH cartridges[15,19]. Each cartridge was extracted by solid phase extraction (SPE) with 4 mL of acetonitrile and brought to a final volume of 5 mL with acetonitrile[20]. Subsequently, each extract was analyzed by HPLC/UV (365 nm) on a Deltabond Res AK column (4.6 mm x 25 cm, Keystone). The mobile phase consisted of (A) 45:55 acetonitrile:water and (B) 75:25 acetonitrile:water, using a 30 minute gradient from A to B and held at B for 5 minutes at a flow rate of 1 mL/min. Each cartridge was extracted by solid phase extraction (SPE) with 4 mL of acetonitrile and brought to a final volume of 5 mL with acetonitrile. Instrument calibration was accomplished using solutions prepared from a purchased aldehyde/ketone DNPH mix solution (15 µg/mL as formaldehyde, Supelco 47285-U) in acetonitrile. A six-point calibration curve was prepared with analyte amounts ranging from 0.0109 to 2.175 µg/ml. Individual carbonyls (formaldehyde, acetaldehyde, acetone, proprionaldehyde, crotonaldehyde, butyraldehyde, benzaldehyde, iso-valeraldehyde, valeraldehyde, o- RTI International/EPA December 2010 A - 3 tolualdehyde, m-tolualdehyde, p-tolualdehyde, hexanaldehyde, 2,5-dimethylbenzaldehyde) were quantitated against the curve and were corrected for amounts found in blank samples. Total carbonyls were computed by summing the individual carbonyl species.

2.7 Results and Discussion

The test method was utilized to analyze the microbial resistance and product VOC offgassing of 3 separate building materials. These materials varied and consisted of a rolled insulation, a spray insulation, and a flooring material. The sections below provide a summary of the individual materials tested and the results of the testing. Due to the rigorousness and completeness of this analysis, Georgia Pacific has utilized this test method as a marketing plan to emphasize the microbial resistant qualities of their different wallboard products, specifically addressing the 12 week total testing time and the rigorousness of the test http://www.buildgp.com/newsRelease.aspx?NewsID=8108.

3.0 Lonwood Natural Flooring

3.1 Test Material

The following description of the product was provided by the vendor and was not verified.

Lonwood Natural flooring is a sheet vinyl product with an embossed wood-grain texture. Constructed in multiple layers and embossed with distinctive wood grains, it is composed of resin, plasticizers, fillers, and pigments. The co-calendered wear layer is formulated to provide maximum resistance to foot traffic in most commercial and healthcare applications. The middle layer provides dimensional stability, sound-absorbing properties, and resiliency under foot. The backing layer provides strength and stability of the flooring and enhances the bonding strength of the adhesive. Mold resistance is conveyed by the addition of a proprietary chemical as a top layer formulation that is applied to the surface of the sheet vinyl through a calendering process. Figures 2 and 3 show the front and back surfaces of the material.

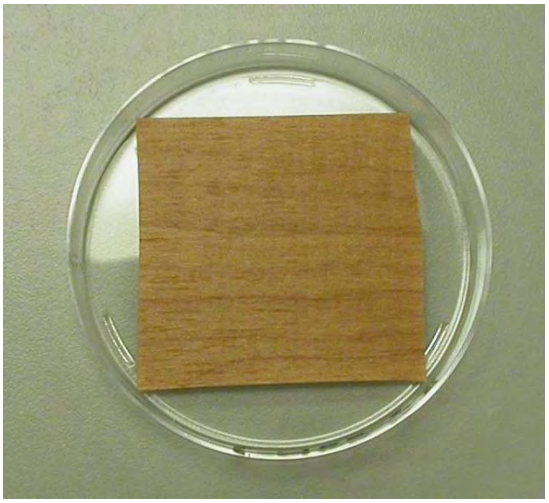

Figure 2. Front surface of material

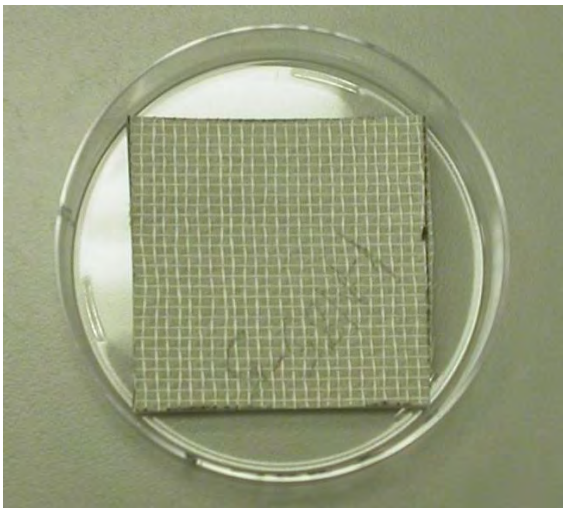

Figure 3. Back surface of material

3.2 Mold Resistance

The results for the mold resistance tests are shown in Table 1. Growth is measured by culture and is defined as at least a 1 $\log_{10}$ increase in culturable organism over the baseline which was determined on Day 0.

Table 1. Log_{10} CFUs for test material (Lonseal) and reference material (wood) on each test date (Mean ± SD)

Lonseal			
Week	***A. versicolor* 85% ERH**	***S. chartarum* 100% ERH**	**Growth of Naturally Occurring Fungi 100% ERH**
0	5.0 ± 0.1	5.0 ± 0.04	< 2.2 ± 0.0*
1	4.8 ± 0.1	NA	4.8 ± 0.6
6	4.4 ± 0.1	NA	6.0 ± 0.1
7	4.2 ± 0.01	NA	6.2 ± 0.2
12	4.1 ± 0.1	NA	6.4 ± 0.3
Reference Material			
Week	***A. versicolor* 85% ERH**	***S. chartarum* 100% ERH**	**Growth of Naturally Occurring Fungi 100% ERH**
0	4.9 ± 0.1	4.8 ± 0.1	< 2.2 ± 0.0*
1	4.7 ± 0.1	3.9 ± 0.2	2.6 ± 1.0
6	4.3 ± 0.2	NA	6.3 ± 0.0
7	4.1 ± 0.1	NA	7.0 ± 0.2
12	5.5 ± 0.4	NA	6.9 ± 0.3

NA = Not Available due to overgrowth by innate fungi * = < 2.2 indicates 0 CFU detected at the minimum detection limit

The numbers of CFUs on each test and reference piece were Log_{10} transformed and the mean and standard deviation calculated. The initial concentration is in the row labeled week 0 (day 0 inoculum). The results for the test organisms, *A. versicolor* and *S. chartarum*, are in columns two and three. The fourth column gives the CFUs for the fungi that were on the unsterilized surface of the test material at the initiation of the test.

At Day 0 the numbers of naturally occurring fungi were below the detection limit on both the test and the reference materials. However, the growth of a variety of fungal species (naturally occurring on the sample) was masking any *S. chartarum* growth on Lonseal and on the reference material (wood).

Figure 4 shows the log change in *A. versicolor* and Figure 5 shows the log change in the naturally occurring fungi that are on the surface of the material.

Neither the test material nor the reference material inoculated with *A. versicolor* and incubated at 85% ERH showed growth during the 12 weeks of the test. It was important to check that none of the changes made to the test material to make it mold resistant actually enhanced the ability of mold to grow over the positive control material[11]

It was not possible to accurately assess whether or not the test material was resistant to the growth of *S. chartarum*. The growth of a variety of fungal species (naturally occurring on the sample) masked any *S. chartarum* growth on Lonseal and on the reference material.

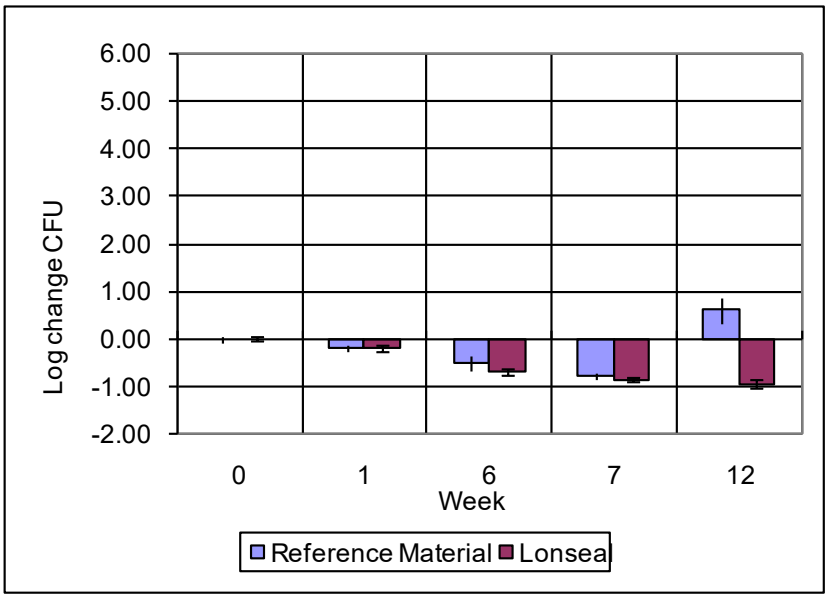

Figure 4. Log change in *Aspergillus versicolor* inoculated on the test material over 12 weeks on the wood reference material and Lonseal.

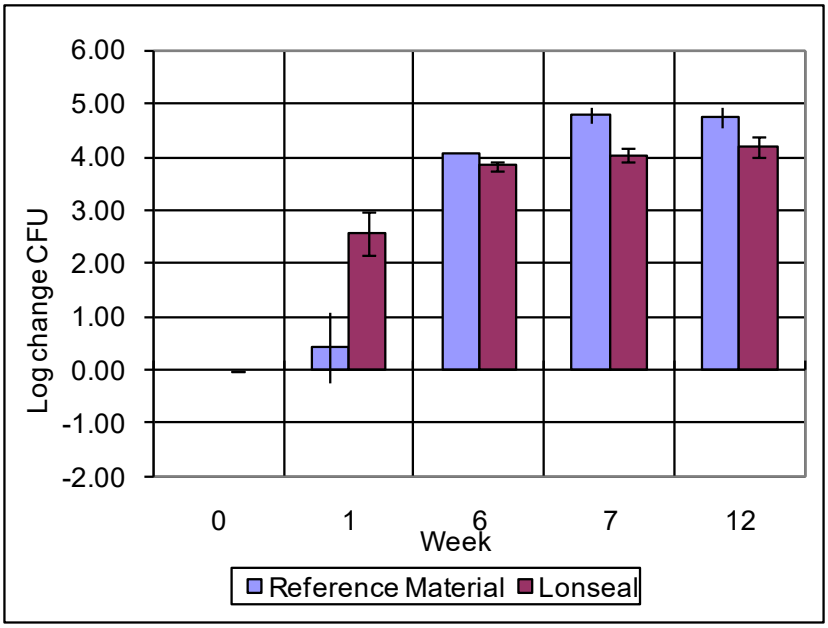

Figure 5. Log change in *naturally occurring fungi* (not inoculated) on the test material over 12 weeks on the wood reference material and Lonseal.

3.3 Emissions of VOCs and Formaldehyde

The emissions of VOCs and formaldehyde test results are presented in the Table 2.

Table 2. Test results for VOCs and formaldehyde emissions from Lonseal

VOCs and Formaldehyde Emissions*	
Emission Types	Minimum emission results
Total VOCs	< 0.5 mg/m^3
Formaldehyde	<0.1 ppm
Individual VOCs	< 0.1 TLV

*Individual pollutants must produce an air concentration level no greater than 1/10 the threshold limit value (TLV) industrial workplace standard (Reference: American Conference of Government Industrial Hygienists, 6500 Glenway, Building D-7, Cincinnati, OH 45211-4438.

3.4 Data Quality Assessment

The quality assurance officer has reviewed the test results and the quality control data and has concluded that the data quality objectives given in the approved Test/QA plan and shown in Table 4 have been attained.

The DQO for the critical measurement, quantitation of fungal growth on an individual test date, is found in Table 3.

Table 3. Data quality objectives

Test	Parameter	DQO		
		Precision	Accuracy	Completeness
Mold Resistance	Quantitation of fungal growth on an individual test date	± 5-fold difference	10% of the plates will be counted by a second operator. ± 20% agreement between the operators	100%

3.5 Emissions Report for Lonseal Flooring Material

Two pieces of Lonseal flooring material, contained in a 7"x7"x2" cradle of aluminum foil, were tested in the small (52.7 L capacity) emissions chamber maintained at 25 ºC and 50% relative humidity and subjected to an air exchange rate of 1 hr^{-1}. After equilibration of each sample for 6 hr[14], sequential samples for VOCs and carbonyls were collected from the chamber effluent for 20 and 120 minutes, yielding collection volumes of approximately 1.5 and 10 L for VOCs and 10 and 60 L for carbonyls[15]. In addition to the test flooring material, replicate chamber blanks and the emission profile of a positive control material (vinyl show curtain liner) were collected. All sample collections and analyses were

conducted in accordance with RTI's AIHA quality manual guidelines.[16]

VOC samples were collected on Carbopack B cartridges. A total of 100 ng of the internal standard, d8-toluene, was subsequently added to each cartridge by flash loading[17] prior to analysis by thermal desorption-GC/MS on a DB-5 column programmed from 40ºC - 225ºC at 5 ºC/min[18]. Calibration standards were prepared at two levels (3.5 µg; 6.9 ng) by flash loading of a 26-component VOC mixture (ethanol; isopropanol; acetone; dichloromethane; carbon disulfide; methyl –t –butyl ether; 2-butanone; 1,1,1-trichloroethane; 1-butanol; trichloroethene, 4-methyl-2-pentanone; toluene; hexanal; tetrachloroethene; m-xylene; n-nonane; 2-butoxyethanol; phenol; 1,2,4-trimethylbenzene; n-decane; 2-ethyl-1-hexanol; d-limonene; 1,2-dichlorobenzene; n-undecane; decamethylcyclosiloxane; n-dodecane) plus d_9-toluene internal standard in methylene chloride onto Carbopack B. In addition to quantitation of the individual analytes, total VOCs (TVOC) were determined by summing the integrated peak areas in the samples and blanks between the retention times of hexane and hexadecane. Two specific analytes, 4-phenylcyclohexene and styrene, were sought in each sample. Neither compound was detected in the samples or blanks. All detected analytes were quantitated against the toluene peak in the standards. No mathematical correction for the blanks was performed.

Carbonyl samples were collected on DNPH cartridges.[15,19] Each cartridge was extracted by solid phase extraction (SPE) with 4 mL of acetonitrile and brought to a final volume of 5 mL with acetonitrile[20]. Subsequently, each extract was analyzed by HPLC/UV (365 nm) on a Deltabond Res AK column (4.6 mm x 25 cm, Keystone). The mobile phase consisted of (A) 45:55 acetonitrile:water and (B) 75:25 acetonitrile:water, using a 30 minute gradient from A to B and held at B for 5 minutes at a flow rate of 1 mL/min. Each cartridge was extracted by solid phase extraction (SPE) with 4 mL of acetonitrile and brought to a final volume of 5 mL with acetonitrile. Instrument calibration was accomplished using solutions prepared from a purchased aldehyde/ketone DNPH mix solution (15 µg/mL as formaldehyde, Supelco 47285-U) in acetonitrile. A six-point calibration curve was prepared with analyte amounts ranging from 0.0109 to 2.175 µg/ml. Individual carbonyls (formaldehyde, acetaldehyde, acetone, proprionaldehyde, crotonaldehyde, butyraldehyde, benzaldehyde, iso-valeraldehyde, valeraldehyde, o-tolualdehyde, m-tolualdehyde, p-tolualdehyde, hexanaldehyde, 2,5-dimethylbenzaldehyde) were quantitated against the curve and were corrected for amounts found in blank samples. Total carbonyls were computed by summing the individual carbonyl species.

The results of the emission tests for VOCs and carbonyls are presented in Tables 4 and 5, respectively. For all samples, excluding the positive control, levels of VOCs and carbonyls were extremely small, near the detection limit for the method, and comparable to the levels found in the blanks.

Table 4. VOC emission results[a] for Lonseal flooring material

Sample Id.	Toluene Chamber Conc. (mg/m^3)	TVOC Chamber Conc. (mg/m^3)	Toluene Emission Factor ($mg/m^2 \cdot hr$)	TVOC Emission Factor ($mg/m^2 \cdot hr$)
Chamber Blank[b]	0.009 (0.005)	0.25 (0.116)	0.015 (0.008)	0.43 (0.20)
Positive Control[c]	0.017 (0.007)	14.2 (1.1)	0.029 (0.012)	23.6 (1.8)
Lonseal flooring[d]	0.003 (0.003)	0.27 (0.13)	0.006 (0.005)	0.46 (0.43)

[a] Mean (Standard deviation) [b] Mean of 3 determinations [c] Mean of 2 determinations [d] Mean of 6 determinations

Table 5. Carbonyl emission results[a] for Lonseal flooring material.

Sample Id.	Formaldehyde Chamber Conc. (mg/m^3)	Total Carbonyls Chamber Conc. (mg/m^3)	Formaldehyde Emission Factor ($mg/m^2 \cdot hr$)	Total Carbonyls Emission Factor ($mg/m^2 \cdot hr$)
Chamber Blank[b]	<0.001	0.017 (0.013)	<0.001	0.028 (0.023)
Positive Control[b]	<0.001	0.012 (0.013)	<0.001	0.021 (0.022)
Lonseal flooring[c]	0.001 (0.002)	0.015 (0.012)	0.003 (0.004)	0.026 (0.021)

[a] Mean (Standard deviation) [b] Mean of 2 determinations [c] Mean of 6 determinations

4.0 Amerrock Premium Plus Rockwool Insulation

4.1 Test Material

The following description of the product was provided by the vendor and was not verified.

Amerrock Premium Plus™ Rockwool insulation is a 100% natural spray insulation. It is made from trap rock and steel slag and contains no chemicals other than annealing oil for dust suppression. When sprayed in place, the interlocking fibers permanently bond to the sheathing material. Premium Plus™ insulation is used in new and existing construction in both the exterior and interior walls.

Figure 6 shows a representative piece of the material.

Figure 6. Premium Plus™ Rockwool Insulation

4.2 Mold Resistance

The results for the mold resistance tests are shown in Table 6. Growth is measured by culture and is defined as at least a 1 $\log_{10}$ increase in culturable organism over the baseline which was determined on Day 0.

Table 6. $\log_{10}$ CFUs for test material (Amerrock) and reference material (insulation) on each test date (Mean ± SD)

Amerrock			
Week	***A. versicolor* 85% ERH**	***S. chartarum* 100% ERH**	**Growth of Naturally Occurring Fungi 100% ERH**
0	5.0 ± 0.1	5.2 ± 0.0	NG
1	4.9 ± 0.1	5.3 ± 0.1	NG
6	4.7 ± 0.2	5.1 ± 0.1	NG
12	4.4 ± 0.7	5.0 ± 0.1	NG
Reference Material			
Week	***A. versicolor* 85% ERH**	***S. chartarum* 100% ERH**	**Growth of Naturally Occurring Fungi**

			100% ERH
0	5.0 ± 0.1	5.2 ± 0.0	3.3 ± 0.2
1	4.5 ± 0.3	5.2 ± 0.1	3.9 ± 0.6
6	3.2 ± 0.0	4.8 ± 0.4	5.4 ± 1.5
12	3.9 ± 1.1	3.7 ± 0.9	5.0 ± 0.9

NG = No Growth

The numbers of CFUs on each test and reference piece were Log_{10} transformed and the mean and standard deviation calculated. The initial concentration is in the row labeled week 0 (day 0 inoculum). The results for the test organisms, *A. versicolor* and *S. chartarum* are in columns two and three. The fourth column gives the CFUs for the fungi (naturally occurring) that were on the unsterilized surface of the reference material at the initiation of the test.

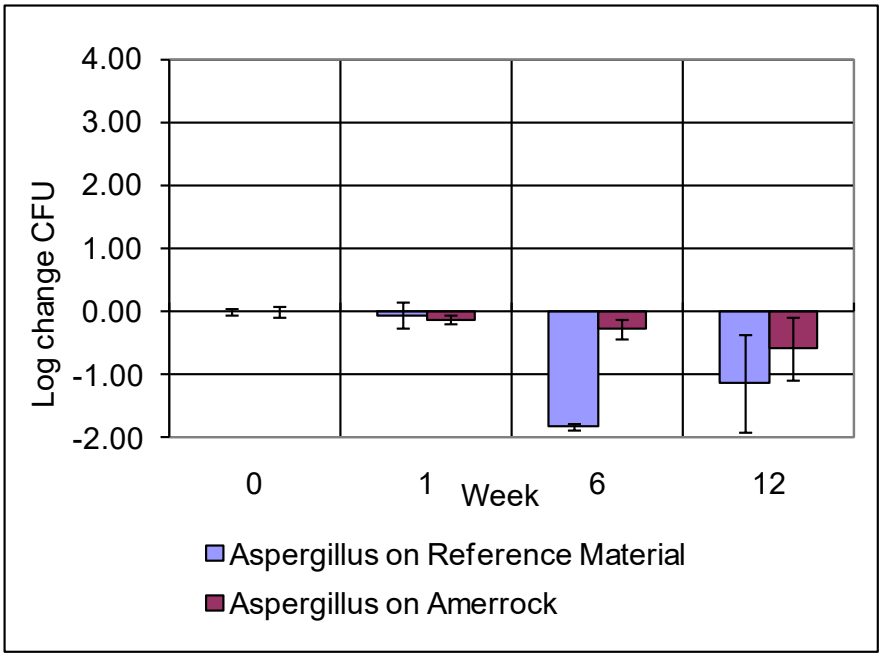

Figure 7. Log change in *Aspergillus versicolor* inoculated on the test material over 12 weeks on the insulation reference material and Amerrock.

Figure 7 shows the log change in *A. versicolor* and Figure 8 shows the log change in *Stachybotrys chartarum* on both the test and reference materials as well as the growth of naturally occurring fungi on the reference material.

Neither the test material nor the reference material inoculated with *A. versicolor* and incubated at 85% ERH showed growth during the 12 weeks of the test.

Neither the test material nor the reference material inoculated with *S. chartarum* and incubated at 100% ERH showed growth during the 12 weeks of the test. The growth of a variety of fungal species on some pieces (naturally occurring on the sample) made it difficult to accurately assess the *S. chartarum* growth on the reference material.

At Day 0 the numbers of naturally occurring fungi were below the detection limit on both the test and the reference materials. However, the growth of the naturally occurring fungi on the reference material became a notable quantity by week 6.

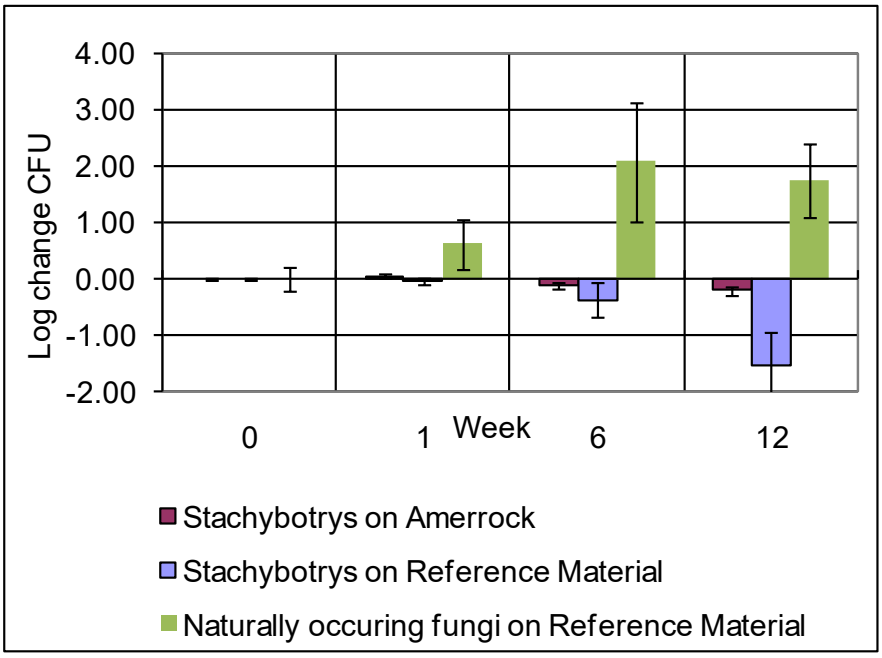

Figure 8. Log change in *Stachybotrys chartarum* inoculated on the test material over 12 weeks on the insulation reference material and Amerrock.

4.3 Emissions of VOCs and Formaldehyde

The emissions of VOCs and formaldehyde test results are presented in the Table 7.

Table 7. Test results for VOCs and formaldehyde emissions from Amerrock

VOCs and Formaldehyde Emissions*	
Emission Types	Minimum emission results
Total VOCs	< 0.5 mg/m^3
Formaldehyde	<0.1 ppm
Individual VOCs	< 0.1 TLV

*Individual pollutants must produce an air concentration level no greater than 1/10 the threshold limit value (TLV) industrial workplace standard (Reference: American Conference of Government Industrial Hygienists, 6500 Glenway, Building D-7, Cincinnati, OH 45211-4438.

4.4 Data Quality Assessment

The DQO for the critical measurement, quantitation of fungal growth on an individual test date, is found in Table 8.

Table 8. Data quality objectives

Test	Parameter	DQO		
		Precision	Accuracy	Completeness
Mold Resistance	Quantitation of fungal growth on an individual test date	± 5-fold difference	10% of the plates will be counted by a second operator. ± 20% agreement between the operators	100%

4.5 EMISSIONS REPORT FOR AMERROCK ROCKWOOL INSULATION

A single 7"x7"x1.5" bed (40 g) of Amerrock® insulation, contained in a 7"x7"x2" cradle of aluminum foil, was tested in the small (52.7 L capacity) emissions chamber maintained at 25ºC and 50% relative humidity and subjected to an air exchange rate of 1 hr^{-1}. After equilibration of the sample for 6 hr[14], sequential samples for VOCs and carbonyls were collected from the chamber effluent for 20 and 120 minutes, yielding collection volumes of approximately 1.5 and 10 L for VOCs and 10 and 60 L for carbonyls[15]. In addition to the test material, a chamber blank and emissions from a positive control material (vinyl show curtain liner) were also collected. All sample collections and analyses were conducted in accordance with RTI's AIHA quality manual guidelines and approved by the EPA project quality management plan.[16]

VOC samples were collected on Carbopack B cartridges. A total of 100 ng of the internal standard, d8-toluene, was subsequently added to each cartridge by flash loading[17] prior to analysis by thermal desorption GC/MS on a DB-5 column programmed from 40º-225º at 5º/min[18]. Calibration standards were prepared at two levels by flash loading of a nine-component VOC mixture plus internal standard in methylene chloride onto Carbopack B. In addition to quantitation of the individual analytes, total VOCs (TVOC) were determined by summing the integrated peak areas in the samples and blanks between the retention times of hexane and hexadecane. Two specific analytes, 4-phenylcyclohexene and styrene, were sought in each sample. Neither compound was detected in the samples or blanks. All detected analytes were quantitated against the toluene peak in the standards. No mathematical correction for the blanks was performed.

Carbonyl samples were collected on DNPH cartridges[15]. Each cartridge was extracted by solid phase

extraction (SPE) with 4 mL of acetonitrile and brought to a final volume of 5 mL with acetonitrile[19]. Subsequently, each extract was analyzed by HPLC/UV (365 nm) on a Supelcosil™ LC-18 column (Supelco #358298, 25 cm x 4.6 mm). The mobile phase consisted of (A) 45:55 acetonitrile:water and (B) 75:25 acetonitrile:water, using a 30 minute gradient from A to B and held at B for 5 minutes at a flow rate of 1 mL/min. Instrument calibration was accomplished using solutions prepared from a purchased aldehyde/ketone DNPH mix solution (15 µg/mL as formaldehyde, Supelco 47285-U) in acetonitrile. A six-point calibration curve was prepared with analyte amounts ranging from 18.8 to 600 ng/mL. Individual carbonyls were quantitated against the curve and corrected for blanks.

The results of the emission tests for VOCs and carbonyls are presented in Tables 9 and 10, respectively. For all samples, excluding the positive control, levels of VOCs and carbonyls were extremely small, near the detection limit for the method, and comparable to the levels found in the blanks.

Table 9. VOC emission results for Amerrock Premium Plus™ Rockwool Insulation

Sample Id.	Toluene Chamber Conc. (mg/m^3)	TVOC Chamber Conc. (mg/m^3)	Toluene Emission Factor ($mg/m^2 \cdot hr$)	TVOC Emission Factor ($mg/m^2 \cdot hr$)
Chamber Blank[a]	<0.001	0.024	<0.001	0.039
Positive Control[a]	<0.001	0.438	<0.001	0.771
Amerrock insulation[b]	<0.001	0.027 (0.019)	<0.001	0.048 (0.035)

[a] Single determination [b] Mean of 7 determinations (standard deviation)

Table 10. Carbonyl emission results for Amerrock Premium Plus™ Rockwool Insulation

Sample Id.	Formaldehyde Chamber Conc. (mg/m^3)	Total Carbonyls Chamber Conc. (mg/m^3)	Formaldehyde Emission Factor ($mg/m^2 \cdot hr$)	Total Carbonyls Emission Factor ($mg/m^2 \cdot hr$)
Chamber Blank[a]	<0.001	<0.001	<0.001	<0.001
Positive Control[a]	<0.001	0.014	<0.001	0.024
Amerrock insulation[b]	<0.001	<0.001	<0.001	<0.001

[a] Single determination [b] Mean of 7 determinations

5.0 AP Armaflex Roll Insulation

5.1 Test Material

The following description of the product was provided by the vendor and was not verified.

AP Armaflex Roll Insulation is a black flexible closed-cell, fiber-free elastomeric thermal insulation. It is furnished with a smooth skin on one side which forms the outer exposed insulation surface. The expanded closed-cell structure makes it an efficient insulation for ductwork, large piping, fittings, tanks and vessels. AP Armaflex products are made with Microban® antimicrobial product protection for added defense against mold on the insulation.

Figures 9 and 10 show the top and bottom surfaces of the material.

Figure 9. Top (outer) surface of material

Figure 10. Bottom (inner) surface of material

5.2 Mold Resistance

The results for the mold resistance tests are shown in Table 11. Growth is measured by culture and is defined as at least a 1 $\log_{10}$ increase in culturable organism over the baseline which was determined on Day 0.

Table 11. Log_{10} CFUs for test material (Armacell) and reference material (insulation) on each test date (Mean ± SD)

Armacell			
Week	***A. versicolor***	***S. chartarum***	**Growth of Naturally**

	85% ERH	*100% ERH*	Occurring Fungi 100% ERH
0	4.5 ± 0.3	5.1 ± 0.1	NG
1	4.1 ± 0.2	3.5 ± 0.8	NG
6	3.1 ± 0.3	3.5 ± 0.3	NG
12	3.0 ± 0.2	3.3 ± 0.4	NG
Reference Material			
Week	***A. versicolor* 85% ERH**	***S. chartarum* 100% ERH**	**Growth of Naturally Occurring Fungi 100% ERH**
0	4.6 ± 0.4	5.0 ± 0.2	< 3.2 ± 0.0*
1	3.8 ± 0.3	5.0 ± 0.1	< 3.2 ± 0.0*
6	3.2 ± 0.3	4.3 ± 1.0	4.8 ± 2.0
12	3.0 ± 0.5	4.2 ± 0.9	4.9 ± 2.3

NG = No Growth * = < 3.2 indicates 0 CFU detected at the minimum detection limit

The numbers of CFUs on each test and reference piece were Log_{10} transformed and the mean and standard deviation calculated. The initial concentration is in the row labeled week 0 (day 0 inoculum). The results for the test organisms, *A. versicolor* and *S. chartarum* are in columns two and three. The fourth column gives the CFUs for the fungi (naturally occurring) that were on the unsterilized surface of the reference material at the initiation of the test.

Figure 11 shows the log change in *A. versicolor* and Figure 12 shows the log change in *Stachybotrys chartarum* on both the test and reference materials as well as the growth of naturally occurring fungi on the reference material.

Neither the test material nor the reference material inoculated with *A. versicolor* and incubated at 85% ERH showed growth during the 12 weeks of the test. It was important to check that none of the changes made to the test material to make it mold resistant actually enhanced the ability of mold to grow over the positive control material[11]

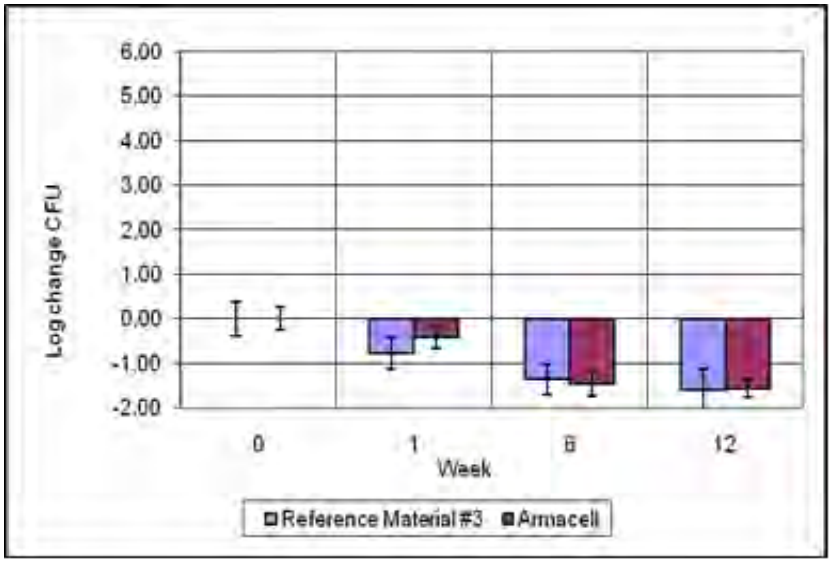

Figure 11. Log change in *Aspergillus versicolor* inoculated on the test material over 12 weeks on the insulation reference material and Armacell.

Neither the test material nor the reference material inoculated with *S. chartarum* and incubated at 100% ERH showed growth during the 12 weeks of the test. The growth of a variety of fungal species on some pieces (naturally occurring on the sample) made it difficult to accurately assess the *S. chartarum* growth on the reference material due to crowding out the *S. chartarum* and making it difficult to detect.

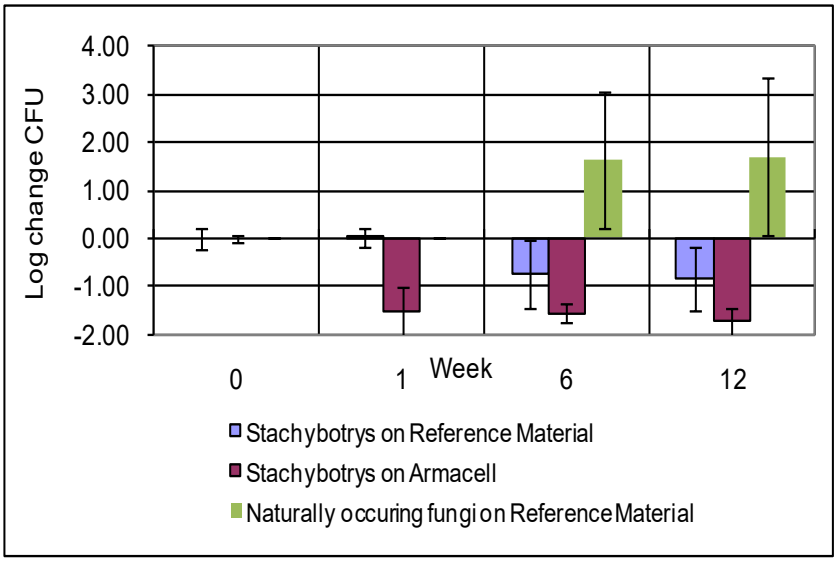

Figure 12. Log change in *Stachybotrys chartarum* inoculated on the test material over 12 weeks on the insulation reference material and Armacell.

At Day 0 the numbers of naturally occurring fungi were below the detection limit on both the test and the reference materials. However, the growth of the naturally occurring fungi on the reference material became a notable quantity by week 6 on the reference material.

5.3 Emissions of VOCs and Formaldehyde

The emissions of VOCs and formaldehyde test results are presented in the Table 12.

Table 12. Test results for VOCs and formaldehyde emissions from Armacell

VOCs and Formaldehyde Emissions*	
Emission Types	Minimum emission results
Total VOCs	< 0.5 mg/m^3
Formaldehyde	<0.1 ppm
Individual VOCs	< 0.1 TLV

*Individual pollutants must produce an air concentration level no greater than 1/10 the threshold limit value (TLV) industrial workplace standard (Reference: American Conference of Government Industrial Hygienists, 6500 Glenway, Building D-7, Cincinnati, OH 45211-4438.

5.4 Data Quality Assessment

The quality assurance officer has reviewed the test results and the quality control data and has concluded that the data quality objectives given in the approved Test/QA plan and shown in Table 4 have been attained.

The DQO for the critical measurement, quantitation of fungal growth on an individual test date, is found in Table 13.

Table 13. Data quality objectives

Test	Parameter	DQO		
		Precision	Accuracy	Completeness
Mold Resistance	Quantitation of fungal growth on an individual test date	± 5-fold difference	10% of the plates will be counted by a second operator. ± 20% agreement between the operators	100%

5.5 EMISSIONS REPORT FOR AP ARMAFLEX BLACK MATERIAL

A single 7"x7" sample of AP Armaflex Black material was tested in the small (52.7 L capacity) emissions chamber subjected to an air exchange rate of 1 hr^{-1}. After equilibration of the sample for 6 hr, sequential samples for VOCs and carbonyls were collected from the chamber effluent for 20 and 120 minutes, yielding collection volumes of approximately 1.5 and 10 L for VOCs and 10 and 60 L for carbonyls. In addition to the test material, a chamber blank and emissions from a positive control material (vinyl show curtain liner) were also collected.

VOC samples were collected on Carbopack B cartridges and were analyzed by GC/MS on a DB-5 column programmed from 40º-225º at 5º/min. Calibration standards were prepared at two levels by flash loading of a VOC mixture in methylene chloride onto Carbopack B. In addition to quantitation of the individual analytes, total VOCs (TVOC) were determined by summing the integrated peak areas in the samples and blanks between the retention times of hexane and hexadecane. Two specific analytes, 4-phenylcyclohexene and styrene, were sought in each sample. Neither compound was detected in the samples or blanks. All detected analytes were quantitated against the toluene peak in the standards. No mathematical correction for the blanks was performed.

Carbonyl samples were collected on DNPH cartridges and were analyzed by HPLC/UV (365 nm) on a Supelcosil™ LC-18 column (Supelco #358298, 25 cm x 4.6 mm). The mobile phase consisted of (A) 45:55 acetonitrile:water and (B) 75:25 acetonitrile:water, using a 30 minute gradient from A to B and held at B for 5 minutes at a flow rate of 1 mL/min. Each cartridge was extracted by solid phase extraction (SPE) with 4 mL of acetonitrile and brought to a final volume of 5 mL with acetonitrile. Instrument calibration was accomplished using solutions prepared from a purchased aldehyde/ketone DNPH mix solution (15 µg/mL as formaldehyde, Supelco 47285-U) in acetonitrile. A six-point calibration curve was prepared with analyte amounts ranging from 18.8 to 600 ng/mL. Individual carbonyls were quantitated against the curve and corrected for blanks.

The results of the emission tests for VOCs and carbonyls are presented in Tables 14 and 15, respectively. For all samples, excluding the positive control, levels of VOCs and carbonyls were extremely small, near the detection limit for the method, and comparable to the levels found in the blanks.

Table 14. VOC emission results[a] for AP Armaflex Black® Material

Sample Id.	Toluene Chamber Conc. (mg/m^3)	TVOC Chamber Conc. (mg/m^3)	Toluene Emission Factor ($mg/m^2 \cdot hr$)	TVOC Emission Factor ($mg/m^2 \cdot hr$)
Chamber Blank[b]	<0.001	0.0470	0.0007	0.0829
Positive Control[b]	0.000	0.6708	0.000	1.1600
AP Armaflex Black[c]	<0.001	0.042 (0.030)	<0.001	0.074 (0.053)

[a] Mean (Standard deviation) [b] Single determination [c] Mean of 6 determinations

Table 15. Carbonyl emission results[a] for AP Armaflex Black® Material

Sample Id.	Formaldehyde Chamber Conc. (mg/m^3)	Total Carbonyls Chamber Conc. (mg/m^3)	Formaldehyde Emission Factor ($mg/m^2 \cdot hr$)	Total Carbonyls Emission Factor ($mg/m^2 \cdot hr$)
Chamber Blank[b]	<0.001	0.004	<0.001	0.007
Positive Control[b]	<0.001	0.013	<0.001	0.023
AP Armaflex Black[c]	0.001 (0.003)	0.012 (0.010)	0.002 (0.006)	0.021 (0.019)

[a] Mean (Standard deviation) [b] Single determination [c] Mean of 6 determinations

Testing of microbial resistance coupled to product VOC offgassing utilizing quantitative endpoints is a major step forward in the analysis of products utilized in the built environment. These methods allow for the direct comparison between products and for the selection of the product that best meets the desired needs of the end user. The quantitative analysis and longer total testing period add robustness to the results allowing repeatability and confidence in the results. This robustness can be seen in the analysis that follows which compares this method with the other available and utilized testing protocols.

6.0 METHOD ANALYSIS OF MICROBIAL-RESISTANT GYPSUM PRODUCTS

Introduction: There have been numerous estimates that humans spend approximately 90% of their time in the indoor environment[21, 22]. With such a large amount of time spent inside, it is clear that environmental conditions within the built environment can adversely affect human health[22]. An increasingly important aspect of indoor environmental quality is the impact biological organisms, mainly the filamentous fungi (mold) have on adverse human health. Estimates of fungal contamination in the indoor environment in North America range between 20% to 40%[23, 24]. The presence of fungi in the indoor environment may play a role in "Sick Building Syndrome[25] leading to health effects including itchy eyes, fatigue, headache, and possibly idiopathic pulmonary hemosiderosis in infants resulting in death[26, 27, 28, 29, 30, 31, 32, 33]. A major component of the interior of buildings, as well as being a potential growth substrate for fungal organisms is gypsum wallboard.

Numerous companies within the gypsum industry have recognized the need to limit fungal growth in the indoor environment and have taken the lead to develop gypsum wallboard products that are resistant to fungal growth. These companies have gone about making their products microbial resistant in different ways. One such technique has been the removal of all paper and cellulosic adhesives from their microbial resistant gypsum products, thereby eliminating the nutrient source (food) for the fungi to grow. This methodology has replaced the paper backing with fiberglass matting. Another company has utilized a different method of producing fungal resistant gypsum products. Their products consist of the addition of a fungicide into both the core and the paper of their products. The main fungicide utilized in these products is sodium pyrithione. Sodium pyrithione is a broad spectrum and highly efficient antimicrobial. It has been used to control bacteria, fungi, yeast and algae. Additional benefits of sodium pyrithione are that it does not produce VOCs and maintains good environmental stability. Similarly, another company has introduced a different antimicrobial into the paper of their gypsum products. This antimicrobial is Sporgard WB. Sporgard WB is actually a combination of 3 different fungicides acting together. Azoxystrobin, thiabendazole, and fludioxonil combine in Sporgard WB to synergistically inhibit the growth of fungal organisms on paper gypsum surfaces. Despite these advances however, there is no nationally accepted testing and verification methodology to guide consumers and building professionals on how to select or specify the best gypsum products for their needs. There are numerous methods available to test gypsum products for microbial resistance and this manuscript details a comparative analysis of the different methods currently in use.

During previous test method development efforts in the US Environmental Protection Agency microbial resistant gypsum wallboard project, a common theme from both stakeholders and product vendors was the need for a unified and accepted method of testing gypsum products that was both accurate and repeatable. There are a number of methods currently used to test for microbial resistance. Some are quantitative, but most are not. The objective of this study was to evaluate currently utilized microbial (fungal) resistant testing methodologies as applied to gypsum products. The available test methods were reviewed through a literature search and through the product information of the gypsum board material claims. The literature search included, but was not limited to, EPA and ASTM methods. The methods selected were: (1) EPA for mold-resistant gypsum board testing[34, 35]; (2) ASTM D 3273 - Resistance to growth of mold on the surface of interior coatings in an environmental chamber[36]; (3) ASTM D 2020 – Mildew resistance of paper and paperboard[37]; (4) ASTM C 1338 – Standard test method for determining fungi resistance of insulation materials and facings[38]; and (5) ASTM G 21- Standard practice for

determining resistance of synthetic polymeric materials to fungi[39].

Our study provides a comparison of the most commonly used current methods and will allow for industry uniformity when comparing the microbial resistance efficacy of individual products. Likewise, it enables vendors and testing laboratories to choose the proper analytical method for testing their products.

Materials and Methods: The available test methods were reviewed by the EPA through a literature search and through the product information of the gypsum board material claims. The five test methods selected have all been used in mold-resistant claims by at least 1 manufacturer for at least 1 gypsum panel material. Each of the methods were reviewed by the EPA in detail and performed by RTI as specified in each of the different method procedures. The following describes the test materials and the individual test methods that were compared beginning with the EPA method.Table 1 summarizes and compares each of the methods for some key specifications.

Gypsum wallboards. Four different trade mark gypsum wallboard products were purchased locally at retail stores. Of the four gypsum boards selected, only two had biocide incorporated in the product either added to the paper lining or to the paper lining and the gypsum core. As mentioned before, the purpose of this study was to compare methods and for this reason the gypsum products trademarks were omitted. These were represented as follows: W1; W2; W3 and W4. The uniformity of the test materials was maximized by obtaining a sufficient quantity of each material so that any irregularities that occurred during the manufacturing process were compensated for by random selection of all pieces cut from a particular source.

EPA mold-resistant gypsum board testing[35]. The EPA method addresses two specific characteristics of mold-resistant building material: 1) mold resistance, and 2) emissions of VOCs and aldehydes. Mold resistance is the critical measurement, so the protocol described is focusing exclusively for this testing. Emissions of VOCs and aldehydes are ancillary tests and may or may not be performed depending upon the relevance to the test material. Other characteristics, such as fire resistance, are important and should be considered by users of the products, but are beyond the scope of this test plan. The EPA mold-resistance testing method followed the guidelines outlined in ASTM D 6329: "Standard guide for developing methodology for evaluating the ability of indoor materials to support microbial growth using static environmental chambers"[34]. This method utilizes small static chambers to evaluate the potential for microbial growth on materials usually found in indoor settings. Clear plastic desiccators served as the static environmental chambers (Figure 2). The desiccators have gasket-sealed doors, which eliminate air exchange and serve to maintain the humidity within the chamber and prevent contamination of the materials by environmental organisms. The chamber humidity was maintained through the use of saturated salt solutions (ASTM E104-02)[40]. Temperature was externally controlled and maintained at room temperature. The chambers were set to the required Equilibrium Relative Humidity (ERH). The ERH in each chamber was monitored with a hygrometer.

Preparation of mold spore suspensions. Mold spore suspensions were prepared using pure cultures of *Stachybotrys chartarum* (RTI 3075) and *Aspergillus versicolor* (RTI 3348). The spores' suspensions were prepared by inoculating the test organism onto Sabouraud dextrose agar (SDA) (Fisher Scientific, Pittsburgh, PA), and incubating the culture at room temperature for 5 – 7 days or until heavy sporulation was observed. A spore suspension was prepared by wiping the spores from the surface of the SDA plate

and eluting into sterile18-Mohm distilled water to a known spore concentration to serve as a stock culture. The stock spore suspension was serially diluted in sterile, 18-Mohm distilled water to a concentration of approximately $10^5 - 10^6$ colony forming units (CFU)/ml. The organism preparation was viewed microscopically to verify purity of spores (spores only, absence of hyphae).

Inoculation and incubation of wallboard coupons. Small (at least 4 cm x 4 cm), replicate coupons of wallboard were prepared and inoculated. Each piece was placed on a separate labeled sterile petri dish. The test pieces were inoculated (usually with five 10µL spots in an X configuration) by pipet directly onto the surface of the wallboard test piece and allowed to dry in a biosafety cabinet before transferring to the corresponding static chamber. The goal was to load each of the individual test pieces with approximately 10^4 to 10^5 CFU/piece.

All of the pieces for one material and one test organism were put in the same static chamber. The chambers were set to100% RH for the tests with *S. chartarum* and at 85% for *A. versicolor*. The tests ran for 12 weeks. Within the 12 weeks of the test, four test dates—Day 0, Week 1, Week 6, and Week 12—were evaluated. Day 0 provided the baseline (inoculum level). To minimize error and demonstrate reproducibility, five pieces of each sample type were processed on each respective sampling day. Because there were four test dates, a minimum of 20 pieces were prepared simultaneously. Each piece was placed on a separate labeled, sterile Petri dish. On each test day (including Day 0), five replicates of the test material pieces were removed from the chamber, placed in sterile buffer, and extracted by shaking. The resulting suspension of eluted organisms was plated on SDA and incubated for 5 – 7 days. Mold growth was determined by manually enumerating colony-forming units (CFUs), counting fungal colonies on the media plate. On each test day (including day 0), the test pieces were removed from the static chamber, placed in approximately 30 mL sterile buffer and extracted by shaking using a vortex or wrist action shaker. Determination of mold growth: The effectiveness of the gypsum products to inactivate the culturable test organisms was quantified by calculating the $\log_{10}$ change in CFU. First, the $\log_{10}$ CFU per coupon was determined. Next, the average and standard deviation of either the replicate positive control coupons (at day 1 prior to incubation) or the replicate inoculated exposed coupons (after 12 weeks of incubation) were calculated. Finally, the log change was calculated as follows:

$$\log_{10} \text{change} = \log_{10} CFU_C - \log_{10} CFU_E \qquad \text{(Eq. 1)}$$

where:

$\log_{10} CFU_C$ = mean $\log_{10}$ CFU of positive control coupons at day 1 prior to incubation.
$\log_{10} CFU_E$ = mean $\log_{10}$ CFU of exposed coupons after 12 weeks of incubation

The uncertainty of the efficacy was calculated using the standard deviations from both the exposed and positive control coupons to determine the combined standard error of the difference for each test.

ASTM D3273: Resistance to growth of mold on the surface of interior coatings in an environmental chamber[36]**.** This method is used to evaluate in a 4-week period the relative resistance of paint films to surface mold fungi and mildew growth in a severe interior environment. This method can be used to evaluate the comparative resistance of interior coating to accelerated mildew growth

(Figure 2).

Determination of mold growth: Rate the panels for mold growth each week for 4 weeks on a visual rating scale of 1 (disfigured) — 10 (no growth) using photographic standards

ASTM D2020: Mildew resistance of paper and paperboard[37]**.** This test method is composed of two methods: a direct inoculation method for materials that are expected to be in damp, warm atmosphere, but not in contact with soil; and a burial method for materials that may be in contact with damp soil for long periods of time (Figure 3).

The direct inoculation method covers the qualitative determination of mildew resistance of paper and paperboard. The direct inoculation, pure culture, nonsterile specimen method is applicable to paper products that are expected to be used or stored in a damp, warm atmosphere, but out of contact with damp soil.

The burial method covers the qualitative determination of mildew resistance of paper and paperboard. This test method is used for papers with or without fungus-resistant treatment, which may be in contact with damp soil for long periods of time. Determination of mold growth: visual rating scale within an incubation period of 14 days (2 weeks). Test gypsum materials that showed growth after 7 or 14 days incubation were reported as not resistant. Test gypsum materials that showed no growth after 14 days incubation were reported as resistant.

ASTM C1338: Standard test method for determining fungi resistance of insulation materials and facings[38]**.** This test method is used to determine the relative ability of an insulation and its facing to support or resist fungal growth under conditions favorable for their development. This test method uses a comparative material to determine the relative ability of a material to support fungal growth (Figure 4).

Determination of mold growth: visual rating scale within an incubation period of 28 days. A rating of pass or failed was used for interpretation of results. Test materials that showed no mold growth within the incubation period were scored as passed and those that showed mold growth were scored as failed.

ASTM G 21: Standard practice for determining resistance of synthetic polymeric materials to fungi[39]**.** This test method covers the determination of the effects of fungi on the properties of synthetic polymeric materials in the form of molded and fabricated articles, tubes, rods, sheets, and film materials (Figure 6).

Determination of mold growth consisted of visual examination of the gypsum material after 28 days of incubation. The following rating was used: no growth = 0; traces of growth = 1; light growth = 2; medium growth = 3; heavy growth = 4. Microscopic examination of the material required to confirm ratings of trace or no growth.

Table 16. Overview of each of the test methods and showing a comparison of selected key parameters and specification

	EPA ETV-ESTE: ASTM D6329	ASTM D3273	ASTM D2020 –	ASTM C1338	ASTM G21
Apparatus	Chamber	Constructed chamber	Chamber or room	Chamber	Incubator/chamber
Conditions	Based on appropriateness for the environment where material is used	32.5 ± 1 °C 95 - 98% RH	28 ± 1 °C Humid preferred	30 ± 2 °C 95 ± 4% RH	28 - 30 °C ≥ 85% RH
Test Organism	*Aspergillus versicolor* (RTI 3348) *Stachybotrys chartarum* (RTI 3075) (ASTM D6329 – organisms not specified; based	*Aureobasidium pullulans* (ATCC 9348) *Aspergillus niger* (ATCC 6275) *Penicillium citrinum* (ATCC 9849)	*Chaetomium globosum* (ATCC 6205) *Aspergillus terreus* (ATCC 7860) *Aspergillus niger* (ATCC 9642)	*Aspergillus niger* (ATCC 9642) *A.versicolor* (ATCC 11730) *Penicillium brevicompactum (RTI 3495*) *Chaetomium*	*A. niger* (ATCC 9642) *P. brevicompactum (RTI 3495)* *Chaetomium globosum* (ATCC 6205) *Gliocladium virens* (ATCC 9645)
Inocula	Culture spores	Culture spores	Culture spores and mycelia	Culture spores	Culture spores
Inoculation Method	Single organism suspension directly onto surface of material and allowed to dry	Inoculation soil; hang panels over soil	Direct inoculation of specimen on nutrient-salts agar plate	Atomize 0.5 mL onto specimen	Atomize onto specimen on nutrient-salts agar plate
Length of	12 weeks	4 weeks	2 weeks	4 weeks	4 weeks unless growth detected sooner
Controls	Reference building material purchased from a retail store;	Ponderosa pine	Similar untreated material	White birch tongue depressor or related material	Filter paper
Results	Quantitative rating scale	Visual Qualitative rating scale	Visual examination	Visual Pass/Fail relative to control under	Visual Qualitative rating scale

6.1 Results and Discussion:

Removal of growth substrates or the incorporation of antimicrobial agents in the manufacturing of gypsum products may prevent mold growth and the spread of biological contaminants. The potential for a material to be mold resistant can be assessed in the laboratory using standard tests. However, there is no accepted testing method to guide consumers and building professionals on how to select or specify the best gypsum products for their needs[41]. In this study, we evaluated five currently utilized microbial (fungal) resistant testing methodologies in the search for a method that is both accurate and repeatable when applied to gypsum products. It is clear from looking at Table 1 that these methods use multiple different organisms, have different lengths, and different conditions. This makes it very confusing when deciding which method to use to test a product. The development of a test method that can be used to test numerous different product classes with a sufficiently long test duration (12 weeks) and a quantitative endpoint were needed to standardize microbial resistant product testing.

Table 2 summarizes the results for each gypsum material utilized - W1, W2, W3, W4 - following each test method. The four different materials are listed in the last four columns of the table. Each test is given its own section, with the interpretation of the result for each material following the method. Of the five methods compared, only the EPA Environmental Technology Evaluation-Environmental and Sustainable Technology Evaluation (ETV-ESTE) gave a quantitative endpoint; all of the others had a qualitative endpoint. All of the methodologies evaluated showed that gypsum material W1 was the least mold-resistant. Gypsum material W2 showed to be mold-resistant when using the qualitative methods, however, the quantitative test EPA ETV-ESTE, showed that *Stachybotrys chartarum* grows on W2 when incubated at 100%RH and room temperature within a period of 12 weeks of incubation. All the qualitative methodologies showed that W3 was mold-resistant (the W3 material was not tested following the EPA ETV-ESTE protocol). One of our major findings was with product W4. All the qualitative methods showed that W4 was mold resistant. However, when using the EPA ETV-ESTE protocol, it was shown that the naturally occurring mycobiota showed growth within an incubation period of 6 weeks (data not shown). This comparison study demonstrated that longer incubation periods are necessary for testing of mold-resistant gypsum products since the naturally occurring mycobiota is undetected with shorter incubation periods.

The EPA ETV-ESTE testing for mold resistant test is based on the ASTM D 6329 guidelines. It allows the testing of gypsum materials under real world scenarios to evaluate its mold-resistance performance and the results are measurable. On the other hand, qualitative methodologies rely on visual ratings which is subject to misinterpretation.

Our study provides a comparison of the most commonly used current methods and will allow for industry uniformity when comparing the microbial resistance efficacy of individual products. Likewise, it enables vendors and testing laboratories to choose the proper analytical method for testing their products. Development of this test method was carried out due to the lack of a standard methodology to test numerous different microbial resistant building product classes. It improves upon the numerous methods already in use by extending the testing duration and utilizing a quantitative endpoint.

Table 17. Summary of test results for each of the gypsum panel materials

EPA ETV-ESTE					
		Log_{10} change of CFU (± standard error of the mean)			
Aspergillus versicolor – 85% RH	12 weeks	0.4 ± 0.2	-1.7 ± 0.2	N/A	-0.3 ± 0.5
Growth of naturally occurring mycobiota @ 85% RH	12 weeks	No growth	No growth	N/A	No growth
Stachybotrys chartarum @ 100% RH	12 weeks	Overgrown	0.2 ± 0.5	N/A	-1.6 ± 0.4
Growth of naturally occurring mycobiota @100% RH	12 weeks	4.5 ± 0.3	No growth	N/A	1.8 ± 0.7
ASTM D3273					
	Incubation	Rating Range visual scale of 1 (disfigured) — 10 (no growth)			
Aureobasidium pullulans	4 weeks	*6*	10 (no growth)	6	10 (no growth)
Aspergillus niger	4 weeks	*6*	10 (no growth)	6	10 (no growth)
Penicillium citrinum	4 weeks	*6*	10 (no growth)	6	10 (no growth)
ASTM D2020					
	Incubation	**Visual examination***			
Chaetomium globosum	7 Days	NR	R	NR	R
	14 Days	NR	R	NR	R
Aspergillus terrus	7 Days	NR	R	NR	R
	14 Days	NR	R	NR	R
Aspergillus niger	7 Days	NR	R	NR	R
	14 Days	NR	R	NR	R
Control - no fungi inoculated	7 Days	NR	R	NR	R
	14 Days	NR	R	NR	R
*NR - Not resistant— test specimens that showed growth after 7 or 14 days incubation					
ASTM C1338					
	Incubation	**Visual examination. Pass/Fail Rating**			
A. versicolor	4 weeks	Fail	Pass	Fail	Pass
A. niger	4 weeks	Fail	Pass	Fail	Pass
P. brevicompactum	4 weeks	Fail	Pass	Fail	Pass
C. globosum	4 weeks	Fail	Pass	Fail	Pass

A. flavus	4 weeks	Fail	Pass	Fail	Pass
ASTM G21					
	Incubation	Visual examination of fungal growth *			
A. niger	4 weeks	2	0	2	0
ASTM G21					
		Visual examination of fungal growth *			
P. brevicompactum	4 weeks	2	0	2	0
C. globosum	4 weeks	2	0	2	0
Gliocladium virens	4 weeks	2	0	2	0
A. pullulans	4 weeks	2	0	2	0
*No growth = 0; Traces of growth = 1; light growth = 2; medium growth = 3; heavy growth= 4					

Figure 13. ASTM D6329. Test chamber and *Stachybotrys* growth on reference material.

Figure 14. ASTM D3273. Inoculated test materials in chamber suspended over inoculated soil.

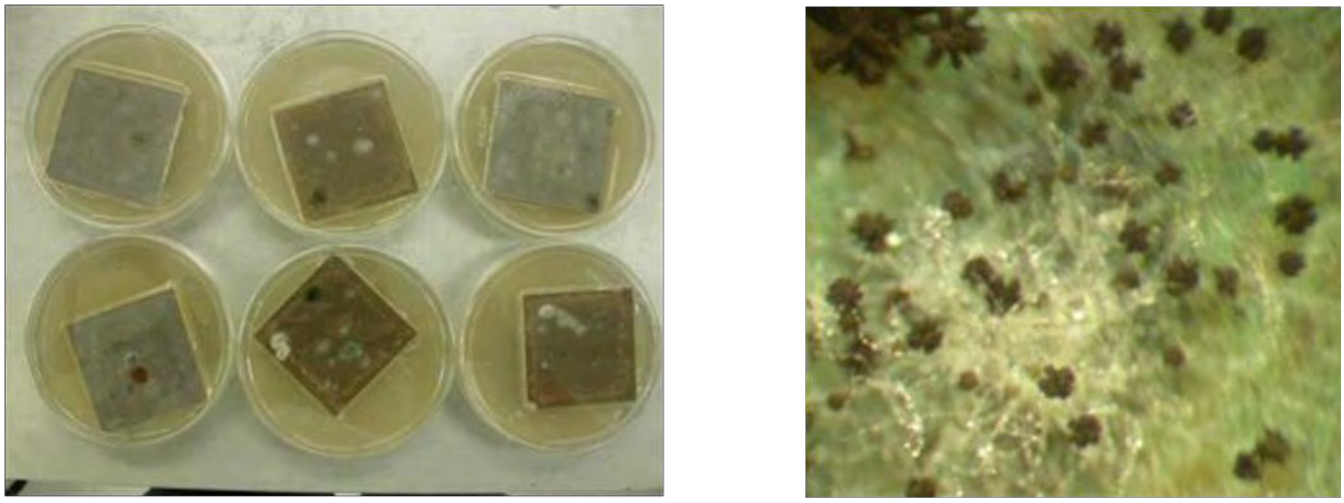

Figure 15. ASTM D2020. Test materials in nutrient agar showing growth.

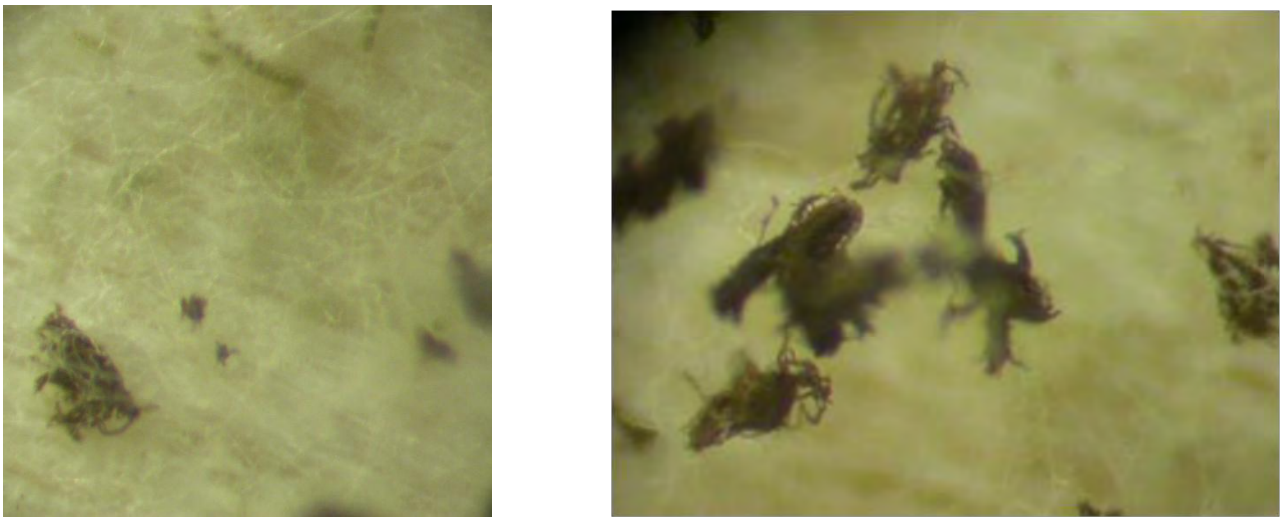

Figure 16. ASTM1338. Comparative material (birch tongue depressor) on left and test material on right.

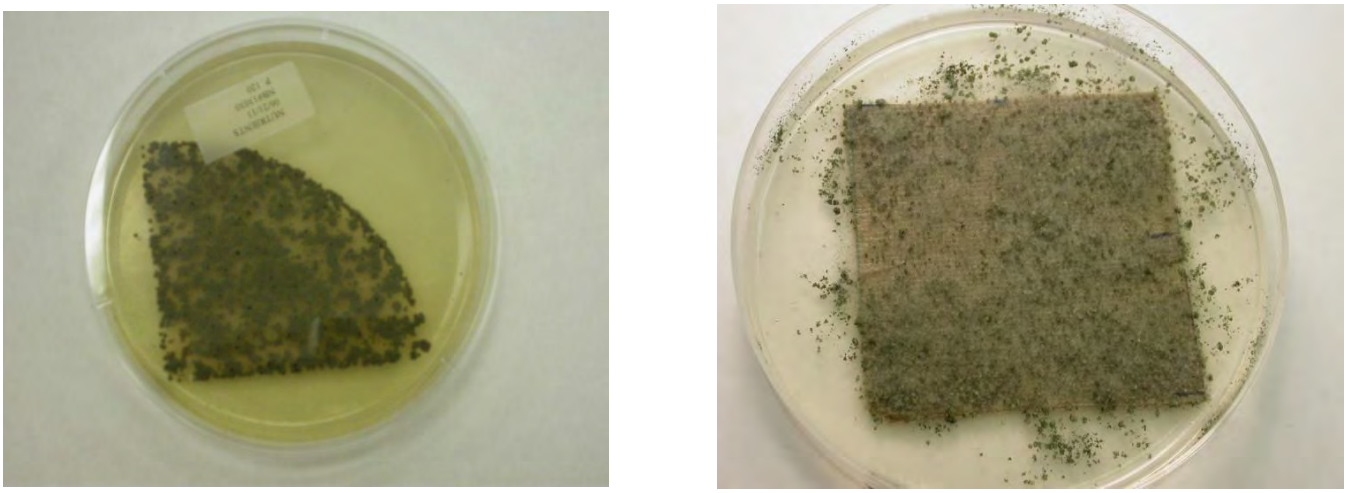

Figure 17. ASTM G21. Reference material (filter paper) on left and test material on right.

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